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Prenatal screening for genetic disorders: Suggested guidelines for the Indian Scenario

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Prenatal testing is the best strategy for reducing the burden of genetic disorders and congenital disabilities that cause significant postnatal functional impairment. Universal prenatal screening is advisable for common genetic disorders and congenital anomalies such as Down syndrome, beta-thalassaemia and neural tube defects. Several prenatal-screening tests are now available for Down syndrome, but knowledge about the appropriate timing of the test and the need for pre- and post-test counselling may not be updated among the primary care physicians. There is also a considerable degree of confusion regarding the prenatal screening test to be chosen in each case, due to the availability of a number of new and advanced screening techniques. At present, there is no nation-wide consensus regarding the nature and timing of these prenatal-screening protocols. Due to the absence of any definite guidelines and the additional lacunae in the awareness regarding the appropriate prenatal screening in the country, the optimum benefits of these screening protocols are not reaching the population. This review focuses on the various prenatal screening and diagnostic tests that are available for common genetic conditions and congenital disabilities and attempts to outline the most cost-effective and gestational age-appropriate strategies for prenatal screening for the Indian healthcare set-up. The recommendations suggested would serve as a source guide for formulating prenatal-screening guidelines for reducing the incidence of common genetic disorders and congenital disabilities in India.

Key words Beta thalassaemia - Down syndrome - India - neural tube defects - prenatal-screening guidelines

Introduction

Prenatal diagnosis for major genetic disorders and congenital disabilities with a poor prognosis and discontinuation of the pregnancy if the foetus is affected, is an accepted strategy for reducing the burden of genetic disorders. At-risk families are mostly identified after the birth of an affected child or an informative family history and offered appropriate genetic counselling with the option of prenatal diagnosis for the condition under consideration. However, many genetic disorders occur in families without any history of an affected child or individual. With advances in medical science, screening tests have become available for the prevention of common genetic

disorders and are being offered to all pregnant women. The disorders with a significant prevalence in India for which population-based prevention programmes are needed include beta-thalassaemia, Down syndrome and neural tube defects (NTDs)[1-4]. Screening and prenatal diagnosis for these disorders are available through the public and private sectors in India, and awareness amongst obstetricians and primary care physicians is increasing. However, information about the correct test, appropriate time for ordering the test and expertise for pre-test and post-test counselling is often lacking. The availability of numerous screening options with varied detection rates and costs adds to the inconsistencies in counselling for the appropriate screening option. Many countries such as Canada have national guidelines for Down syndrome screening[5]. In India, a population-based government programme for antenatal screening is not available. However, the need for appropriate, evidence-based screening necessitates that we develop guidelines for prenatal screening to assist obstetricians and primary care providers prevent the birth of an affected child. These guidelines will also help policymakers to plan and initiate a country-wide programme and include prenatal screening as a part of routine antenatal care.

The Indian scenario

There are adequate published data on the prevalence of Down syndrome, beta-thalassaemia and NTDs to appreciate the need for population-based screening for these disorders in India. An approximate of 21,400 children with Down syndrome, 9000 with beta-thalassaemia and 5200 with sickle cell disease are born in India every year[6,7]. However, multiple screening options, variability of economic status and heterogeneity of the prevalent medical services in the country make it a challenge to follow a single uniform screening protocol[8]. This article is aimed to provide information about the multitude of available screening tests and issues to be considered while choosing the appropriate test, along with information for the clinicians to make correct decisions about offering the screening test to the families.

Screening strategies

Beta-thalassaemia

Thalassaemia major is a serious disorder with difficult, costly and life-long treatment. If both partners are carriers of beta-thalassaemia, the risk of birth of a child with thalassaemia major is 25 per cent or one in four. Couples mostly come for counselling for the secondary prevention after the birth of an affected child with homozygous or compound heterozygous beta-thalassaemia (thalassaemia major or thalassaemia intermedia). It is uncommon that they are identified through primary preventive measures such as extended family screening or preconception carrier screening. The carrier frequency of beta-thalassaemia in India is 3-17 per cent in various population groups[1,9]. The carrier frequencies of haemoglobin (Hb) E and S are up to 40 per cent in some regions[10]. Given the high burden of the disease and carrier rate in India, population screening to identify thalassaemia carriers with subsequent counselling and prenatal testing for at-risk families is essential to decrease the disease burden. Population-wide screening has helped to successfully control and reduce the incidence of beta-thalassaemia in Mediterranean countries[11,12].

Screening test and timing of the test for thalassaemia and haemoglobinopathies: The best time to screen couples for thalassaemia and haemoglobinopathies is pre-pregnancy or at the first antenatal visit preferably in the first trimester. This is the time when the couple/families are keenly interested in the well-being of the to-be-born child, more receptive to the information provided and are willing for immediate and appropriate actions. Over-enthusiastic groups have been advising premarital screening and avoidance of marriage between thalassaemia carriers. Such actions affecting social behaviour, usually do not have general acceptance by the population and may also stigmatize individuals causing problems with marriage proposals. Hence, this strategy is not advocated. Screening of adolescents or college-going students has similar implications, and other than creating awareness, the utility of such a strategy is not documented[7,13]. Screening of asymptomatic children for carrier status is not ethically correct and should not be done[14,15].

The screening test recommended for detection of carrier state of beta-thalassaemia and haemoglobinopathies is quantification of HbA2, HbF and other variants such as HbS, HbD and HbQ India by cation exchange high-performance liquid chromatography (CE-HPLC). Decreased red cell indices [mean cell volume (MCV) < 80 fl and mean cell Hb (MCH) < 27 pg] in association with HbA2 ≥ 3.5 per cent will detect most of the beta-thalassaemia carriers. Use of HPLC is essential to detect other Hb variants such as HbS, HbC and HbE as these have clinically significant interactions with beta-thalassaemia. Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) though simple and less costly, is not

sensitive, has a significant error rate and should not be used for population screening. Isolated use of red cell indices, though very useful for beta-thalassaemia, has been found to miss a significant proportion of beta-thalassaemia carriers during pregnancy[16]. Atypical beta-thalassaemia carriers may have a normal MCV and/or MCH sometimes and may be missed if screened using only red blood cells indices. Red cell indices are also normal in a significant proportion of carriers of HbS and HbE[1]. If both the partners are found to be carriers of beta-thalassaemia or haemoglobinopathy, genetic counselling regarding risk of recurrence and prenatal diagnosis is to be provided. Prenatal diagnosis needs identification of mutation in both partners before testing the DNA of the foetus by chorionic villus sampling (CVS). The process takes at least a week or more and the sample has to be sent to a genetics laboratory. Therefore, to allow for timely prenatal diagnosis, pre-pregnancy screening is the best strategy. Till the concept of pre-conception counselling becomes popular and widely available, thalassaemia screening will need to be scheduled in the first antenatal visit in the first trimester, preferably before eight weeks of gestation. In such situations, the husband and wife should be tested simultaneously if possible to avoid delay caused by testing them serially. Feasibility of such a screening programme for beta-thalassaemia has been proved by pilot studies[9,17].

Neural tube defects (NTDs)

NTD is the most common congenital malformation, and its primary and secondary prevention is possible by periconceptional folic acid therapy[18,19]. Food fortification has been successful in decreasing the incidence of NTDs in many countries[20]. However, NTDs still continue to be one of the most common congenital malformations[21]. In a study from India, 62 per cent of the prenatally detected NTDs were found to have been identified after 20 wk of gestation, which is the legally permissible limit of medical termination of pregnancy in India[22]; this delayed detection could be partly attributable to the inconsistency in the timing of the antenatal imaging and lack of adequately trained antenatal sonologists. There is a need for screening of all pregnancies for NTDs at the appropriate gestation, appropriate training of antenatal sonologists for early detection of NTDs and other major anomalies and creating awareness about folic acid intake by women of childbearing age till the country adopts a food fortification policy. Anencephaly detection is possible in 100 per cent cases as early as 12-14 wk gestation. Meningocele, encephalocele and open spina bifida can also be detected by ultrasonographic (USG) evaluation at 16-20 wk gestation, especially if careful attention is paid to the 'lemon' and 'banana' signs which are good pointers to NTDs. Primary prevention for NTD is an established concept. In India, education and motivation of the pregnant women for regular antenatal follow up and also for the primary care provider to develop infrastructure and adopt the standard of care practice for antenatal detection of NTD by ultrasound and mid-trimester maternal serum alpha-foetoprotein (MsAFP) are required[23,24].

Down syndrome

Down syndrome is the most common cause of intellectual disability and accounts for about 15-30 per cent of cases[4,25]. In India, the birth prevalence is reported to vary from one in 1230 to one in 1361[26,27]. Prenatal screening for Down syndrome started with maternal age as a screening tool and over the last two decades has evolved and achieved almost 99 per cent sensitivity. The commonly used screening tests in India are triple test, quadruple test and first-trimester double-marker test with or without nuchal translucency (NT). The detection rate for the first-trimester biochemical screen test with NT is 82 per cent while that for quadruple test is 80 per cent[28]. Combinations such as integrated or sequential testing and additional ultrasound markers such as nasal bone increase the detection rate to 95 per cent[28]. However, the latter combinations increase the cost, cause more anxiety, require more hospital visits, and necessitate detailed counseling as well as expertise in ultrasonography. Though the first-trimester USG-based strategies have good sensitivity in expert hands, these may not suitable for population-based screening.

Cell-free foetal DNA in maternal plasma: Cell-free foetal DNA (cffDNA) in the maternal plasma is the latest test. With cffDNA-based Down syndrome screening (also known as non-invasive prenatal test or NIPT), detection rates of 99 to 100 per cent have been reported[29-31]. However, due to occasional false negative results, false positivity and failure to get a result in about 2-6 per cent cases, NIPT is still considered to be a screening test only[32]. A meta-analysis of published literature of cffDNA testing showed the detection rate of trisomy 21 was 99.0 per cent with the confidence interval of 95 per cent (98.2-99.6%) with false positive rate of 0.08 per cent[31,33,34]. The detection rates were 96.8 per cent and 92.1 per cent for trisomy 18 and

trisomy 13, respectively. Benn *et al*[28] have compiled studies using various methods for NIPT and have shown similar results. In the study by Quezada *et al*[29], two of the 34 foetuses with trisomy 21 were in the 'No results on NIPT' group, indicating the possibility that foetuses detected to have aneuploidy are more likely to need repeat sampling due to test failure[29]. In the same study where cffDNA gave high risk of trisomy 21 or trisomy 18 and the foetus was normal disomic, the foetal DNA fraction was found to be less. In an Australian audit, three of the 27 cffDNA-positive cases spontaneously aborted before CVS[30]. In a study from Japan, on their nationwide one-year experience with NIPT in 7740 women, four were found to be not reportable, and of the 1638 negative cases who were followed up (from the total of 7594 negative cases), there was one false negative trisomy 18[35].

Appropriate pre-test counselling to guide the family to understand the advantages and limitations of NIPT is important. It remains a screening test though with high sensitivity. A positive NIPT screen result still mandates confirmation by invasive testing before any further course is decided upon. Adams *et al*[36] have stressed the need of providing information to the pregnant women about what NIPT can detect and what it cannot.

Diagnostic options for Down syndrome testing: CVS after 11 completed wk, amniocentesis after 16 wk or cordocentesis after 18-20 wk gestation are the methods used to obtain foetal samples for definitive testing. These procedures need training and expertise and have inherent risks of abortion reported to be 0.2-1.3 per cent and 0.1-0.9 per cent for CVS and amniocentesis, respectively[37,38].

Conventionally, chromosomal fluorescent *in situ* hybridization (FISH) for the common five aneuploidies and karyotype analysis is performed. Another technology-driven revolution in chromosomal analysis is cytogenetic microarray (CMA) that is a molecular cytogenetic technique to visualize chromosomes at a very high resolution. In prenatal samples, cytogenetic microarray is considered an option even in foetuses with normal USG evaluation[39,40]. This will help in primary prevention of numerous well-delineated sporadic microdeletion/duplication syndromes. Individually, these are rare but account for a significant proportion of intellectual disability[41]. CMA detects such copy number variations in one per cent of prenatal samples with any indication and 3-4 per cent of foetuses with malformations[42,43]. One limitation of CMA is that it detects some copy number variations of unknown significance in about one per cent of cases making counselling and prognostication difficult[44]. Like all prenatal testing and screening methods, CMA also needs good supportive counselling facilities. CMA has made its place in all invasive prenatal diagnosis and is here to stay and replace traditional karyotyping.

Choice for screening for Down syndrome and NTDs: As mentioned above, the screening options for Down syndrome and NTD have evolved over time. Triple-marker screening is being done in India for many years, and though there is a paucity of published literature about the sensitivity of the test in the Indian scenario, there are some studies that suggest efficacy and detection rates similar to those reported in other countries. Kaur *et al*[45] published a small study on 7400 pregnant women in north India screened with triple-marker test and showed 5.7 per cent screen positivity and detection of seven of eight Down syndrome babies (1 in 925), similar to reports from other countries[28]. A centre in New Delhi providing biochemical screening for Down syndrome calculated that in a city like Delhi where 3.6 lakh deliveries take place every year and 75 per cent women have at least one antenatal visit in the second trimester, second-trimester triple-marker test can prevent birth of 245 Down syndrome babies every year[46]. In a study from western India, 2111 women were investigated by triple-marker screening between 14 and 20 wk of gestation, of whom 224 women were found to be screen positive for trisomy 21 and further on karyotyping of 105 of the screen-positive cases, eight had trisomy 21 and one had mosaic trisomy 21[47]. In another study which reported the two-year data of a referral institute from northern India, in four out of 68 women (4.4%) with triple-test positivity for Down syndrome, amniotic fluid karyotyping was found to show trisomy 21[48].

Certain facts that need to be considered before deciding on the most cost-effective and practically applicable screening strategy for Down syndrome are that first-trimester USG evaluation needs special training, expertise and time, a significant proportion of pregnancies affected with trisomy 21 are spontaneously aborted before 16 wk of gestation, irreversible decisions such as termination of pregnancy are mostly not taken based on the first trimester USG diagnosis of malformations and there is 3-5 per cent risk of genetic disorders/congenital disabilities other than Down syndrome in each pregnancy, some of which are easily detectable in a second-trimester USG. Table I shows

Table I. Comparison of various screening tests for Down syndrome

Test	Detection rate (%) (OAPR)	False positivity (%)	Cost per test* in ₹ (reporting time)	Comments	Diagnosis other than foetuses with trisomy 21
First trimester USG, PAPP-A + fb hCG# at 12 wk	80 (1 in 29)	3	₹ 4000 (+ ₹ 15,000 for those who need invasive testing) (2-7 days for the screening test & 2-4 wk for the confirmatory test)	Early diagnosis Does not screen for NTDs & other malformations for which USG at 18 wk is needed. Some foetuses with trisomy 21 get spontaneously aborted before 16 wk.	Detection of other chromosomal anomalies in cases undergoing invasive foetal sampling and karyotyping.
AFP + hCG + uE3 + InhA along with USG scan for anomalies at 16-18 wk	80 (1 in 30)	3	₹ 4000 (+ ₹ 15,000 for those who need invasive testing) (2-7 days for screening test & for confirmation - 2-7 days for rapid aneuploidy detection test & 2-4 wk for karyotyping)	Two tests can be done in one visit. Easy to train in second-trimester USG.	AFP screening for NTDs. Detection of NTDs & other malformations by USG (3-5%). Detection of other chromosomal anomalies in cases undergoing invasive foetal sampling and karyotyping.
cffDNA at 10 wk	Almost 99-100	0.04	₹ 30,000 (10 days to four weeks followed by confirmatory test in positive cases)	Reduces the need for invasive testing by 95 per cent. Does not screen for NTD & other malformations. USG at 18 wk needed. Some foetuses with trisomy 21 get spontaneously aborted before 16 wk. No result in 2-3 per cent.	Only trisomy 18, trisomy 13 and sex chromosomal abnormalities detected.
AFP + hCG + uE3 + InhA and USG anomaly scan at 16-18 wk with CMA on invasive samples	80	3	₹ 4000 (+ ₹ 30,000 for CMA for those who need invasive testing) (2-7 days for screening test + for confirmation - 2-7 days for rapid test & 2-4 wk for CMA)	Two tests in one visit. Easy to train in second-trimester USG. CMA to be offered to those who can afford the cost.	Detection of NTDs and other malformations (3-5%) Detection of additional sub-microscopic chromosomal abnormalities in 1% of those who undergo invasive testing.
USG & direct amniocentesis** at 16-18 wk & CMA	99	Nil	₹ 1000 + ₹ 30,000 (2-4 wk)	USG should be done especially as MsAFP is not being done	Detection of NTDs and other malformations (3-5%). Detection of additional sub-microscopic chromosomal imbalances (1%).

#Double marker test; *Approximate costs (derived from authors' institutions); **Can be offered for advanced GA or family history of ID with unknown (uninvestigated) cause. Detection rates at 3 per cent false positivity quoted from Benn *et al*., 2013[28]. OAPR, odds of being affected given a positive result; USG, ultrasonography; NTDs, neural tube defects; PAPP-A, pregnancy-associated plasma protein A; AFP, alpha foetoprotein; hCG, human chorionic gonadotropin; fb hCG, free beta subunit of human chorionic gonadotropin; uE3, unconjugated oestriol; InhA, inhibin A; CMA, chromosomal microarray; GA, gestational age; ID, intellectual disability; MsAFP, maternal serum alpha-foetoprotein; cffDNA, cell free foetal DNA

a comparison of the detection rate and false positivity rate of the various screening tests available for Down syndrome, and the additional advantage and estimated cost for each test. In India, majority of the pregnant women seek medical attention in the second trimester[49]. In this case, second-trimester screening with anomaly scan that screens for markers of aneuploidy and NTD along with other malformations is appropriate. In addition, if CMA is done on amniotic fluid obtained for pregnancies with positive second-trimester screening, it will detect other chromosomal aneuploidies and submicroscopic rearrangements in one per cent of cases. Thus, for eight cases of trisomy 21 detected, this strategy will, in addition, detect about four non-Down syndrome chromosomal anomalies. Cost comparison of invasive testing with CMA and that of NIPT can be discussed with the couple. CMA is an appropriate option if the woman is willing to accept the small risk of abortion as all foetuses with trisomy 21 and additionally 3-4 non-trisomy 21 chromosomal abnormalities that also cause intellectual disability are detected for every eight trisomy 21 foetuses identified. As per the American College of Obstetrics and Gynecology (ACOG Committee on Genetics, 2013) recommendations a cytogenetic microarray is an option for those undergoing invasive sampling for prenatal diagnosis[40].

First-trimester screening provides results earlier than second-trimester screening. However, it does not cover screening for NTDs by AFP, and this is a major congenital malformation in India. Still many cases of NTDs are not detected prenatally. A study reported that nine of 12 cases of spina bifida missed antenatally were not screened by MsAFP indicating the important role of biochemical screening for NTD in the second trimester[23]. NT continues to play an important role in the diagnosis of chromosomal anomalies other than that of 13, 18 and 21 and structural malformations in the era of NIPT[50]. First-trimester screening has the additional advantage of looking for other USG markers such as absence of nasal bone, tricuspid regurgitation and ductus venosus flow abnormality in addition to early screening for malformations. In spite of first-trimester detection of malformations, irreversible decisions of termination are usually not taken without confirmation by a follow up USG in the second trimester for most malformations. Though first-trimester USG done by an expert is a useful tool, it may not be suitable for recommendation as a population-based screening strategy. At present more than 60 per cent NTDs are detected after 20 wk and there is an urgent need to impart training in second-trimester USG[22]. Another point that needs to be considered is that a significant proportion of foetuses with trisomy 21 are spontaneously aborted[51,52], and in these cases, the pregnant women unnecessarily have to undergo stress as well as CVS. CffDNA (NIPT) has the advantage of decreasing the need for invasive testing by 95 per cent. However, the issues with NIPT are the high cost of testing and need for further confirmatory testing in screen-positive cases[53]. Table II illustrates the estimated cost-effectiveness for various options of screening for trisomy 21. Table III sums up the suggested protocol for screening for NTDs and Down syndrome.

Special considerations

Availability of variety of screening tests makes counselling and decision making difficult[32]. Certain special situations in antenatal care require special management. These include pregnancies from assisted reproductive techniques, twin pregnancies, and pregnancies occurring after previous recurrent pregnancy loss. Here, families wish to avoid invasive testing and biochemical screening tests may have limitations. In such situations, USG-based screening and NIPT are helpful. However, some families with such precious pregnancies do not wish to do any type of screening and are willing to take the small risk of trisomy 21. Hence, the counselling should be non-directive and the caring physician should be supportive of the family's decision. However, it should be clarified that USG alone cannot rule out trisomy 21 or chromosomal disorders. In case of dizygotic twins, the possibility of both twins having trisomy 21 is extremely rare ($1/1200 \times 1/1200 = 1/ 1440000$)[26,27]. If amniocentesis detects one twin having trisomy 21 and other to be normal disomic, selective termination has risks for the normal foetus and also may be technically difficult at an advanced gestational age. These issues should be discussed in detail before embarking on a screening strategy. If the first-trimester USG detects cystic hygroma or other major malformations such as anencephaly, appropriate decisions such as CVS and karyotyping and/or termination of pregnancy need to be considered.

Technological revolutions such as incorporation of microdeletion syndromes in NIPT[55] and whole genome sequencing from a single foetal cell[56] soon will pose many more prenatal screening options and challenging situations to the families and obstetricians that will need to be addressed on a timed basis. Key points

Table II. Comparison of cost-effectiveness of various options for antenatal testing for trisomy 21/Down syndrome (calculations for 12,000 women screened expecting that 10 foetuses will be Down syndrome)

Strategy	Combined test (first trimester double marker test + USG for GA & NT) + CVS or amniocentesis for karyotyping in screen positive cases	Quadruple test for all + USG* + amniocentesis for karyotyping in screen positive cases	NIPT for all	Quadruple test + USG* + amniocentesis for CMA in screen positive cases	Direct amniocentesis + CMA on all samples
Trisomy 21 detection	8 of 10	8 of 10	10 (almost) of 10	8 of 10	10 of 10
Other chromosomal anomalies associated with ID	Not significant	Not significant	0 to 1 (trisomy13/18)	3.6 (3-4)	120
Total cost in million ₹	53.4	54	360	58.8	360
Cost per trisomy 21 or chromosomal anomaly in million ₹	6.675	6.675	36	5	2.8
Trisomy 21 missed	2	2 of 10	Almost none No results - 240	2 of 10	None
Total unbalanced chromosomal anomalies detected	8	8	10**	12	120
Structural malformations detected	Need confirmation by repeat USG	360	None	360	360
Foetal losses#	1.2 with CVS & <1 (0.6) with amniocentesis	<1 (0.6)	Almost none	<1 (0.6)	12

*USG in second trimester is expected to look for growth, major malformations, nuchal fold thickness and other soft markers for chromosomal disorders; **For detection of 2 trisomy 21 missed by biochemical screening and which can be detected by NIPT, additional ₹300 million are needed; #Calculated based on an abortion rate of 1 in 500 for CVS procedures and 1 in 1000 for amniocentesis procedures. USG, ultrasonography; GA, gestational age; NT, nuchal translucency; CVS, chorionic villus sampling; ID, intellectual disability; CMA, chromosomal microarray; NIPT, non-invasive prenatal testing. *Source*: Refs 28, 32, 37 & 49

Box. Key points to be kept in mind for the success of a prenatal screening programme

(*i*) The aim of screening programmes is to provide information to the would-be parents about how to avoid the birth of a child with a serious genetic disorder or congenital disability and help them to take decisions that suit their socio-economic, family and emotional situation.
(*ii*) If both spouses are carriers of beta-thalassaemia, they are to be counselled for prenatal testing in each pregnancy, irrespective of the result of testing in the previous conception.
(*iii*) It is necessary to understand the advantages and limitations of the available antenatal screening and diagnostic tests, before offering them to the patients.
(*iv*) All available options have to be explained and discussed with the family, but the couple should be allowed to take their own decision and make an informed choice.
(*v*) Pre-test counselling for screening for Down syndrome is essential. Many individuals fail to understand the concept of screening and probability and one needs to give time to make it clear to them.
(*vi*) These screening tests do not evaluate for all genetic disorders.
(*vii*) A negative screening test or normal amniotic fluid karyotype/CMA does not guarantee a normal baby.
(*viii*) The counselling should be non-directive. Some families may not wish to take the screening test or might not want to proceed with an invasive test after a positive screen result.
(*ix*) The tests or protocols being reported under research should not be applied to patient care till these are verified and accepted by the medical community.
(*x*) A detailed family history should be obtained and minimum three-generation pedigree must be drawn to identify families at risk for other genetic disorders.
(*xi*) High-risk pregnancies should be preferably identified in the preconception period, to evaluate the proband to confirm the genetic disorder, offer carrier testing as relevant, for peri-conceptional folic acid supplementation, to control maternal disorders such as diabetes mellitus, to counsel about the teratogenic effects of anticonvulsants/anticoagulants/any other drugs that the woman might be taking and to impart education and awareness for prevention of birth defects.
(*xii*) Surveillance for other pregnancy-related complications should not be forgotten.

CMA, chromosomal microarray

Table III. Suggested screening protocol for neural tube defect and trisomy 21 in the Indian scenario

Test	GA (wk)	Advantages	Limitations	Comments
USG with dating and NT measurement. NT with biochemical screening by PAPP-A & fb hCG may be offered.	12	Confirmation of GA. Major malformation may be detected. Chorionicity of twins.	All malformations are not detected at 12 wk and follow up scan essential at 16-18 wk.	Preferable mode of screening if first visit is in first trimester. NT measurement & scan for malformations need expertise. Those needing invasive testing may be offered CMA in addition to traditional karyotyping with or without QF-PCR/FISH.
AFP + hCG + uE3 + InhA & simultaneous anomaly scan. Amniocentesis & karyotyping* for screen positive cases.	17-18	Malformation scan and quadruple screening in one visit. Some chromosomally abnormal foetuses get spontaneously aborted during first trimester. Amniocentesis is simpler procedure & with very low abortion rate. USG visibility is appropriate & most malformations are picked up around 18 wk. It avoids the need of repeat USG.	Limited time in view of the MTP act that allows termination till 20 wk only for a foetal abnormality.	The cases needing invasive test can be offered CMA on prenatal sample to look for sub-microscopic imbalances. Biochemical screening result needs to be available by 17-18 wk. For screen positive cases, results of confirmatory tests should be available by 20 wk.
cffDNA (NIPT)	10-12, (so that early report is available); can be done later as well. Should be done after NT scan. If NT >3.5 mm (99th centile), then NIPT is not indicated.	Should be offered to precious pregnancies who wish to screen for Down syndrome and wish to avoid invasive testing.	Though very costly, it can detect only aneuploidies of chromosomes 21, 13, 18, X & Y (this needs to be clarified)** Failure rate of 2-3 per cent needs to be conveyed to the family.	USG at 12 wk and around 18 wk for malformation scanning. At the same cost, CMA in amniotic fluid can detect 1 per cent additional chromosomal anomalies.

*Rapid detection tests such as QF-PCR, MLPA or FISH help in providing results quickly. However, these can identify only aneuploidies of 21, 18, 13, X & Y. If only rapid test is done, the family should be counselled that the abnormalities of other chromosomes will not be detected and the risk of such abnormalities is 1 in 160 (0.62%)[54] after negative result of rapid aneuploidy detection test. Hence, preferably karyotyping or CMA also should be ordered; **The sex is not identified unless there is a sex chromosomal abnormality. USG, ultrasonography; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein A; AFP, alpha foetoprotein; hCG, human chorionic gonadotropin; fb hCG, free beta subunit of human chorionic gonadotropin; uE3, unconjugated oestriol; InhA, inhibin A; CMA, chromosomal microarray; QF-PCR, quantitative fluorescent polymerase chain reaction; FISH, fluorescence *in situ* hybridization; MTP, medical termination of pregnancy; NIPT, non-invasive prenatal testing; cffDNA, cell free foetal DNA; MLPA, multiplex ligation-dependent probe amplification; GA, gestational age

which need to be considered for a successful prenatal programme are given in the Box.

Conclusions

This article aims to help develop guidelines for appropriate screening and prevention of the common genetic disorders/congenital disabilities in India, namely beta-thalassaemia (and other common haemoglobinopathies), NTDs and Down syndrome. Screening for carrier status for beta-thalassaemia and other haemoglobinopathies should be offered to all couples, irrespective of the family history, through Hb HPLC at the preconception stage or in the first antenatal visit, for primary prevention of beta-thalassaemia and haemoglobinopathies. Folic acid (0.4 mg per day daily) needs to be started in the preconception period for all

women of childbearing age for primary prevention of NTDs. The appropriate test for prenatal screening of Down syndrome for each patient would depend on the gestational age at consultation and the detection rate and error rate of the test. First-trimester NT scan and double-marker testing (combined screen) for screening for trisomy 21 may be offered to women who come for consultation in the first trimester. For women who come for their first antenatal visit in the second trimester, a quadruple-marker test should be offered at 16 wk gestation. A first-trimester scan is important for gestational age estimation and for identification of twin gestation and chorionicity. In the second trimester, USG evaluation at 16-20 wk for foetal soft markers of aneuploidy and foetal malformations should be offered to all pregnant women. For pregnancies that screen positive for foetal aneuploidy, amniocentesis and foetal karyotyping should be offered. If invasive testing is being done for pregnancy, cytogenetic microarray testing can be offered after cost discussions. The role of NIPT for aneuploidies in routine screening is still debatable and its true cost-effectiveness needs to be calculated before its introduction in the screening programme. For all screening and diagnostic tests that are performed, accurate pre- and post-test genetic counselling in simple, easily understandable language is essential.

These proposed recommendations would serve as a source guide for formulating prenatal screening guidelines for reducing the incidence of common genetic disorders and congenital disabilities in India.

***Conflicts of Interest*:** None.

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